Effects of BAS 500 06 F on Honeybees Under Semi-Field Exposure Conditions

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Chemical:	Pyraclostrobin
PC Codes:	099100
CAS #:	175013-18-0
MRID:	49459602
EPA Guideline:	850.3040, (EPPO 170)
GLP Statement:	This study was conducted in compliance with OECD Principles of Good Laboratory Practice (1997)
Classification:	The study is classified as "**Acceptable**" and may be used in risk assessments.
Date of Study Completion:	November 23, 2012

EPA Primary Reviewer:	Meghan Radtke, Ph.D., Biologist	Signature: Date: 4/15/15	
EPA Secondary Reviewer:	Ryan Mroz, Biologist	Signature: Date: 4/15/15	

Summary
The objective of the study was to determine the effects of BAS 500 06 F (19% pyraclostrobin), applied as foliar spray on *Phacelia tanacetifolia*, on honeybee mortality and brood development. Overall, no adverse effects of pyraclostrobin exposure were observed.

Honeybees (*Apis mellifera carnica* P.) were supplied from a beekeeper in Leipzig/Rehbach, Germany. They were queen-right, healthy (examined for Varroa mites, foulbrood, and nosema), normal in size (4,100 to 7600 worker bees), and bred from sister queens. Each colony had one body containing 11 combs, including 4 to 9 brood combs and 8 to 10 combs with food.

A farmer's field in Cunnersdorf, Germany was selected for the study. The field was comprised of meadowland that had not received pesticide applications for the previous 3 years. There were three treatment groups: negative control, test item (pyraclostrobin), and reference item (fenoxycarb). The meadowland was sown with *Phacelia tanacetifolia*. At full flowering, strips within the enclosed tunnel structure were treated using a plot sprayer with one foliar application of tap water (control), pyraclostrobin (treatment – 2.2 lb ai/A), and fenoxycarb (reference – 2.7 lb ai/A) on June 14, 2011 during bee flight between 10:40 am and 12:20 pm. Hives were protected from direct spray by a plastic sheet. One hive was used per tunnel (18 m x 6 m) and there were four tunnels per treatment group. Hives were introduced into the tunnels of flowering *Phacelia* 3 days before exposure and then allowed to remain inside of the tunnel 7 days post application. On the evening of June 21, 2011, they were transferred to a nearby forest without flowering main crops or intensive agriculture in Neualtenbach, Germany for the remainder of the 28 day observation period (July 12, 2011).

Statistical analyses were conducted on mortality (number of dead bees per day), foraging activity (number of foraging bees per m^2), brood termination rate (%), and brood indices. Easy Assay 4.0 (Ratte 1998) and ToxRat Professional 2.10.05 (Ratte 2010) were used for the analyses.

Adult Mortality
Mortality was measured using dead bee traps at the hive entrance and three gauze sheets placed at different locations within the tunnel. Daily counts of the number of dead bees in the traps were made from 2 days before the exposure until Day 28 of the exposure. Dead bee counts on the gauze sheets were made daily from Day 1 through Day 7; results were pooled from the three gauze sheets for each day. Observations occurred at approximately the same time each day.

There were no statistically significant increases in mortality noted between the treatment group and the negative control, except for Day 8 of the exposure period. There were a few statistically significant differences between the reference item and the negative control on several days during the course of the study. However, overall, the results indicate that there were not any differences in mortality between any of the experimental groups (Table 1). Statistical analyses included two-sided Tukey-test, and one-sided Student-t or Welch-t tests.

Table 1. Adult honeybee mortality (mean ± standard deviation)

Exposure Day	Control (number of dead bees)	Pyraclostrobin (number of dead bees)	Fenoxycarb (number of dead bees)
-2	41.3 ± 22.1	38.0 ± 4.9	37.5 ± 15.1
-1	28.5 ± 11.2	26.8 ± 8.1	32.5 ± 7.0
0 (before app)	26.8 ± 12.4	35.3 ± 11.0	38.3 ± 11.9
0 (after app)	25.5 ± 3.4	23.3 ± 7.2	26.5 ± 3.0
1	29.0 ± 3.2	43.5 ± 25.0	**41.0 ± 9.8***
2	18.3 ± 8.3	22.0 ± 12.4	21.3 ± 6.2
3	15.8 ± 2.1	23.8 ± 13.1	**20.8 ± 2.1***
4	36.0 ± 10.7	41.3 ± 14.8	27.3 ± 9.3
5	35.5 ± 6.9	31.8 ± 12.4	43.5 ± 7.7
6	32.5 ± 12.0	29.8 ± 19.3	26.3 ± 3.4
7	26.8 ± 11.7	21.0 ± 6.8	25.3 ± 6.2
8	17.5 ± 3.7	**27.3 ± 8.5***	20.8 ± 8.5
9	21.5 ± 7.8	24.5 ± 14.8	6.5 ± 3.3
10	6.0 ± 1.2	7.5 ± 9.3	7.8 ± 3.0
11	8.3 ± 4.0	9.8 ± 7.0	10.8 ± 4.5
12	8.0 ± 3.4	7.3 ± 5.5	5.8 ± 2.2
13	4.0 ± 2.9	6.3 ± 5.9	1.8 ± 0.5
14	5.8 ± 5.4	5.8 ± 7.1	1.3 ± 1.0
15	7.0 ± 3.6	5.3 ± 4.6	1.3 ± 1.0
16	16.3 ± 4.3	17.3 ± 7.6	17.8 ± 5.4
17	9.0 ± 3.7	4.8 ± 2.1	4.5 ± 1.7
18	13.3 ± 3.7	11.0 ± 3.7	20.3 ± 9.2
19	20.5 ± 2.5	14.8 ± 5.2	**31.0 ± 7.8***
20	9.8 ± 3.0	10.3 ± 3.4	**21.0 ± 8.1***
21	14.5 ± 2.5	15.0 ± 8.0	13.0 ± 5.4
22	2.8 ± 1.7	3.5 ± 1.7	2.8 ± 0.5
23	14.0 ± 9.9	15.8 ± 5.7	4.0 ± 2.9
24	4.8 ± 2.2	4.5 ± 1.3	4.5 ± 2.5
25	1.8 ± 0.5	1.8 ± 1.0	1.8 ± 0.5
26	3.3 ± 1.7	3.3 ± 1.3	2.5 ± 0.6
27	2.8 ± 0.5	3.5 ± 1.0	3.5 ± 1.0
28	3.3 ± 1.3	1.5 ± 0.6	2.5 ± 0.6
*Statistically significant difference with the negative control ($p < 0.05$)			

Foraging

Three observation areas were marked in each field (1 m^2) and observed each day. The number of bees that were foraging (actually visiting flowers) were documented once per day between the hours of 10:00 am and 3:00 pm. Assessments occurred daily at approximately the same time each day from Day -2 through Day 7 of the exposure period. In addition, on the day of application, observations were also made four times within the first hour after application and

then at 2, 4, and 6 hours after application. On Day 1, three foraging assessments were carried out between 10:30 am and 5:12 pm.

Foraging activity was slightly reduced (but not statistically significantly different) for the first hour immediately following the application of pyraclostrobin in the treatment group, compared with the negative control. Throughout the rest of the observation periods, foraging activity was the same between the treatment and negative control, and reference item and negative control (one-sided Student-t) (Table 2).

Table 2. Foraging activity for adult workers (mean and standard deviation)

Exposure Day	Control (bees/m^2)	Pyraclostrobin (bees/m^2)	Fenoxycarb (bees/m^2)
-2	12.7 ± 2.4	12.8 ± 1.0	13.5 ± 1.3
-1	11.8 ± 1.7	12.9 ± 0.5	11.3 ± 1.0
0 (before app)	12.9 ± 2.0	13.4 ± 1.3	12.3 ± 1.2
0 0:15 h (after app)	11.9 ± 2.2	10.1 ± 2.9	11.9 ± 1.0
0 0:30 h (after app)	13.2 ± 2.9	11.6 ± 2.1	9.9 ± 1.5
0 0:45 h (after app)	12.4 ± 1.1	10.1 ± 2.1	10.3 ± 0.8
0 1:00 h (after app)	12.1 ± 2.3	10.5 ± 2.2	9.8 ± 1.0
0 2:00 h (after app)	13.7 ± 1.7	12.9 ± 0.9	11.6 ± 0.5
0 4:00 h (after app)	13.5 ± 1.3	12.9 ± 1.2	12.2 ± 0.4
0 6:00 h (after app)	11.9 ± 0.9	10.8 ± 0.4	11.2 ± 0.3
1 (morning)	13.6 ± 1.0	14.0 ± 1.2	14.0 ± 0.6
1 (midday)	13.2 ± 1.1	13.1 ± 1.0	13.6 ± 0.3
1 (afternoon)	13.5 ± 1.6	13.2 ± 1.1	13.6 ± 1.1
2	13.3 ± 1.7	13.3 ± 0.8	13.3 ± 0.6
3	12.9 ± 1.0	12.8 ± 0.3	13.2 ± 0.4
4	11.6 ± 0.9	11.4 ± 0.6	11.8 ± 0.8
5	11.5 ± 0.7	11.6 ± 0.7	12.2 ± 0.6
6	12.5 ± 1.0	13.1 ± 0.6	11.9 ± 0.7
7	12.3 ± 0.8	12.6 ± 0.9	11.8 ± 0.6

Condition of the Colonies and Development of the Bee Brood

The condition of the colony and bee brood were assessed on Day -1, Day 4, Day 10, Day 17, Day 22, and Day 28 after the application. The assessments included the pollen and nectar storage area, the area containing eggs, larvae, and capped cells, and the number of bees per colony. A detailed brood assessment was conducted by marking at least 500 egg-containing

cells in each hive and following them with respect to brood development at each brood assessment. Colony strength was estimated using Imdorf et al. (1987) and Imdorf and Gerig (1999). Digital imagery was used for the assessments.

Differences in larval mortality among the test groups were determined visually. No differences in larval mortality were noted between the negative control and the treatment group; the reference item exhibited an increase in mortality compared with the negative control (Table 3).

Table 3. Pupal mortality (mean and standard deviation)

Exposure Day	Control (number of dead bees)	Pyraclostrobin (number of dead bees)	Fenoxycarb (number of dead bees)
-2	0 ± 0	0 ± 0	0 ± 0
-1	0 ± 0	0 ± 0	0 ± 0
0 (before app)	0.3 ± 0.5	0 ± 0	0 ± 0
0 (after app)	0 ± 0	0 ± 0	0 ± 0
1	0 ± 0	0 ± 0	0 ± 0
2	0.5 ± 1	0 ± 0	0 ± 0
3	0 ± 0	0 ± 0	0 ± 0
4	0 ± 0	0 ± 0	0 ± 0
5	0 ± 0	0 ± 0	0.5 ± 1.0
6	0 ± 0	0 ± 0	0 ± 0
7	0 ± 0	0 ± 0	0.3 ± 0.5
8	0 ± 0	0 ± 0	19.3 ± 10.6
9	0.3 ± 0.5	0.5 ± 1.0	20.0 ± 20.2
10	0 ± 0	0.3 ± 0.5	7.8 ± 13.5
11	0.3 ± 0.5	0 ± 0	23.3 ± 23.9
12	0 ± 0	0 ± 0	12.8 ± 8.5
13	0.3 ± 0.5	0 ± 0	6.5 ± 4.4
14	0.3 ± 0.5	0 ± 0	0.3 ± 0.5
15	0 ± 0	0 ± 0	1.0 0.8
16	0.3 ± 0.5	0 ± 0	23.5 ± 14.2
17	0 ± 0	0 ± 0	22.3 ± 9.5
18	0 ± 0	0 ± 0	5.0 ± 3.5
19	2.0 ± 1.4	0.3 ± 0.5	17.0 ± 19.4
20	0.8 ± 1.0	0 ± 0	8.0 ± 9.4
21	1.5 ± 1.9	1.0 ± 0.8	13.5 ± 9.7
22	0.3 ± 0.5	0 ± 0	6.3 ± 4.0
23	0 ± 0	0 ± 0	1.0 ± 1.2
24	0 ± 0	0.5 ± 1.0	3.3 ± 4.0
25	0 ± 0	0 ± 0	0.3 ± 0.5
26	0 ± 0	0 ± 0	0 ± 0
27	0.3 ± 0.5	0 ± 0	3.8 ± 3.3
28	0 ± 0	0 ± 0	0.8 ± 0.5

Colony strength was similar between the treatment and negative control groups and increased throughout the 28-day observation period. The strength of the reference item group fluctuated and only slightly increased during the study (Table 4).

Table 4. Summary of honeybee colony strength (mean and standard deviation)

Assessment Day	Negative Control (bees per colony)	Pyraclostrobin (bees per colony)	Fenoxycarb (bees per colony)
-1	5709 ± 1316	5484 ± 1185	6300 ± 616
4	4922 ± 929	5738 ± 1372	6075 ± 1671
10	6722 ± 1264	6975 ± 1059	8466 ± 2292
17	7959 ± 1357	8972 ± 1522	7931 ± 957
22	7959 ± 969	9563 ± 1187	7144 ± 912
28	8156 ± 1595	8916 ± 310	7481 ± 350

Brood area (sum of comb area occupied by eggs, larvae, and capped cells) increased in the pyraclostrobin treatment group and the negative control; the increases in brood area were comparable. The reference item had a consistently lower brood area starting at Day 4 during the exposure period through Day 28, compared with the negative control.

The brood termination rate (indicator of the number of brood that died at the observation point compared with the initial observation) was similar between the treatment group and the negative control; however the reference item had a much higher brood termination rate at the end of the observation period (Student-t test) (Table 5).

Table 5. Summary of honeybee brood termination rate (mean and standard deviation)

Assessment Day	Negative Control (% termination rate)	Pyraclostrobin (% termination rate)	Fenoxycarb (% termination rate)
4	14.4 ± 14.7	20.2 ± 16.0	25.6 ± 20.8
10	26.0 ± 17.2	26.1 ± 13.3	35.0 ± 19.0
17	26.1 ± 17.2	26.3 ± 13.1	**57.8 ± 17.9***
22	26.2 ± 17.2	27.0 ± 12.6	**57.8 ± 17.9***
*Statistically significant difference with the negative control ($p < 0.05$)			

The brood index (indicator of brood development by tracking the development of brood in individual cells over time) showed continuous brood development in the treatment group that was statistically similar to the negative control (one-sided Student-t test). The reference item group had a lower brood index that was statistically significantly different on Day 17 and 22 (one-sided Student-t test) (Table 6). The brood compensation index (indicator of colony recovery by tracking the growth stage of brood and/or use of the cell for pollen/nectar storage) shows that queens in all groups were responding to brood loss by laying eggs in vacant cells and that this was occurring at a higher rate in the reference item, which experienced more brood loss, than the treatment or negative control (Table 7).

Table 6. Summary of brood indices (mean and standard deviation)

Assessment Day	Negative Control	Pyraclostrobin	Fenoxycarb
0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
4	2.1 ± 0.4	1.9 ± 0.3	1.7 ± 0.4
10	2.9 ± 0.7	3.0 ± 0.5	2.6 ± 0.8
17	3.1 ± 0.8	3.1 ± 0.5	**1.7 ± 0.7***
22	3.7 ± 0.9	3.7 ± 0.6	**2.1 ± 0.3***
*Statistically significant difference with the negative control ($p < 0.05$)			

Table 7. Summary of brood compensation indices (mean and standard deviation)

Assessment Day	Negative Control	Pyraclostrobin	Fenoxycarb
0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
4	2.1 ± 0.4	1.9 ± 0.2	1.7 ± 0.4
10	3.1 ± 0.7	3.2 ± 0.3	2.7 ± 0.8
17	3.4 ± 0.8	3.4 ± 0.3	**1.8 ± 0.8***
22	3.9 ± 0.9	4.1 ± 0.3	**2.4 ± 1.1***
*Statistically significant difference with the negative control ($p < 0.05$)			

Bee Behavior

Sublethal effects (changes in behavior) were monitored daily for 28 days after the initiation of the exposure. Any abnormal behavior (*e.g.*, intoxication, aggression, intensive flight without landing on crop, accumulation of bees at hive entrance, bees no longer producing pollen balls, swarming) was recorded.

There were no abnormal behavior between the control, treatment, and reference groups.

Study Limitations

The exposure duration was 7 days, which should be taken into consideration when using the information in risk assessments. There is some uncertainty about pesticide exposures that could have taken place when the bees were moved to the forest site after the 7 day initial exposure period. The study indicated that the area was generally free of intensive agriculture and blooming crops; however, more specific information was not included. Usually a reference item is selected that is expected to cause toxic effects in honeybees; however, fenoxycarb is classified as "practically non-toxic" to adult honeybees on an acute contact basis. Consequently, it is unclear if the test system design was sufficient to show toxicological effects. Reference items are not usually required for EPA testing, so this does not detract from the study's classification.

References

Imdorf, A., and L. Gerig. 1999. Lehrgang zur Erfassung der Volksstärke, Schweizerisches Zentrum für Bienenforschung.

Imdorfl. A.. G. Buehlmann. L. Gerig. V. Kilchenmann. and H. Wille. 1987. Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in freifliegenden Bienenvölkern. *Apidologie* 18 (2). 137-146.

Ratte. M. ToxRat Professional 2.10.05 (2010): ToxRat Solutions GmbH. Naheweg 15. 52477 Alsdorf. Germany.

Ratte. H.T. Easy Assay 4.0 (1998): Naheweg 15. 52477 Alsdorf. Germany.